Photosynthetic response of wheat to stress induced by *Puccinia recondita* and post-infection drought

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Abstract

To quantify photosynthetic response of wheat to the combination of a fungal brown rust infection and a post-infection drought, four treatments were compared: no stress (control), fungal stress (FS), water stress (WS), and twofold stress (WS×FS). Predawn leaf water potential (Ψwp) was similar in FS and WS treatments over a 3-week period. In the WS treatment, net photosynthetic rate (PN) and stomata CO₂ conductance (gs) diminished concomitantly with a constant intercellular CO₂ concentration (Ci) close to 200 μmol mol⁻¹. In the FS treatment, a reduction of PN occurred with an increase in respiration rate (doubling of the CO₂ compensation concentration) and in Ci but with no water loss modification. Healthy leaves of infected plants (FS) showed a reduction of PN as well, with constant gs and increased Ci. In the twofold stress treatment (WS×FS), leaves showed reduced PN in relation to the lower Ψwp. Deleterious effects of both drought and fungal infection on the final area of leaves and dry matter were additive.

Additional key words: biotic stress; brown rust; intercellular CO₂ concentration; leaf gas exchange; plant growth; respiration rate; stomata conductance; stress interaction; *Triticum aestivum* L.; water stress.

Introduction

Flag leaves of wheat plants infected by *Puccinia recondita* f. sp. *triticci* showed a decrease in the net photosynthetic rate (PN) per leaf area unit and per chlorophyll (Chl) unit as well as a decrease in transpiration rate (McGrath and Pennypacker 1990). Wheat cultivars susceptible to brown rust had reduced PN and increased Chl content, whereas no modification was observed in resistant cultivars (Statler 1988). Changes in PN also exist in healthy leaves of infected plants (Murray and Walters 1992).

The effects of fungal stress on leaf transpiration rate (E) are well documented (Ayres 1981). The decrease in E results from the stomatal closure following infection by pathogens, the intoxicating action upon host cells, the reduction of air spaces by fungal hyphae, and the possible obstruction of vessels and stomata (Duniway and Durbin 1971). Working on wheat flag leaves, McGrath and Pennypacker (1990) have shown that brown rust induces an E reduction before sporulation. After sporulation, water losses from infected tissue increased primarily through epidermal rupture and through stomata that remained open even under dark or dry conditions. The acceleration of water loss by infected tissues can frequently be attributed to epidermal ruptures (Spotts and Ferree 1979, Tissera and Ayres 1986), to inhibition of stomatal closure (Turner and Graniti 1969), to direct loss from the parasite tissues, or increase in host tissue permeability induced by the secretion of enzymes or fungal toxins (Arntzen et al. 1973).

At plant level, the photosynthesis reduction in wheat plants infected by stem rust has been explained by a decrease in leaf area and by a reduction in CO₂ assimilation efficiency (Berghaus and Reisener 1985), by a decrease in Chl content, by an inhibition in photosynthetic phosphorylation, by modifications in the Hill reaction and CO₂ assimilation (Spotts and Ferree 1979) and in translocation of photosynthates (Okwulekie 2000). In various pathogenic systems studied under field conditions, reductions of PN and E at plant level were caused by early defoliation and leaf drying linked to fungal development and resulted in cell damage and death (Shtienberg 1992). In this field study it was shown that the decrease in PN...
or E was not proportional to the corresponding reduction in healthy leaf area due to disease. However, the pattern of plant response to disease was related to the types of trophic relationship.

For biotrophic fungi such as rusts, the equilibrium modification between water absorbed by roots and the transpiration demand depends on the infected leaf area and the type of infection site. As disease progressed, barley leaves infected by *Puccinia hordei* lose their ability to keep a favourable water status (Berryman *et al.* 1991). A rapid decrease in soil water content caused a slight reduction in the water potential of sugar beet leaves infected by *Erysiphe polygoni* when only the first leaf showed disease symptoms. However, a noticeable reduction can occur when symptoms appear on the first to the seventh or eighth leaves (Gordon and Duniway 1982).

On faba bean leaves infected by rust (*Uromyces viciae-faba*), the water potential of diseased leaves was lower than in healthy leaves (Tissera and Ayres 1986). Following epidermal rupture due to rust lesions, the leaf stomata opened and led to a water potential reduction (Paul and Ayres 1984). Under such unfavourable water regime there was a significant limitation in water use efficiency (Balasubramanian and Gaunt 1990).

It is important to know whether the effects of interacting water stress and fungal constraint are additive. For example, a reduction in *P*ₙ and leaf area was noticed on leaves of groundsel plants infected by rust and temporarily water-stressed under laboratory conditions (Paul and Ayres 1984). This issue has been analysed in the field on wheat to quantify the effects of water stress along with leaf blotch and brown rust diseases; this field work was done under temperate climate (Cowan and Van der Wal 1975) as well as under semi-arid conditions (Shtienberg 1990).

Based on leaf gas exchange techniques and plant growth characterisation, our objective was to quantify the response of wheat leaves to the combination of brown rust infection and post-infection drought in comparison to each single stress. To investigate to what extent leaf *P*ₚₙ and growth reductions of infected wheat plants could be explained by the water stress caused by either brown rust or post-infection drought, response curves of *P*ₚₙ to irradiance and CO₂ concentration were used. To interpret experiments on irradiance response curves obtained in different conditions of infection and post-infection drought, a simple analytical model including parameters dependent on predawn leaf water potential was tested.

**Materials and methods**

**Plants:** Wheat (*Triticum aestivum* L. cv. Michigan Amber) seedlings sensitive to brown rust were grown in a climatic chamber. Healthy seeds were sown in 11×11 cm plastic pots (4 seeds per pot). Environment in the growth chamber was favourable to both plant growth and disease development. The temperature was 17±1 °C during the 16-h light period and 15±1 °C during the 8-h dark period. At 0.8 m from the light sources, the spatial mean of radiation was 416±40 μmol m⁻² s⁻¹. Transpiration and irradiation were controlled by weighing pots using load cells (*Scame, AG 30, Annemasse, France*) connected to a data logger (*Campbell, CR7, Shepshed, UK*). This method made it possible to estimate the amounts of irrigation on a daily basis.

**Fungal pathogens and inoculation procedure:** Plants bearing sporulating lesions were produced under the conditions described above (see also Geagea *et al.* 1999). Five leaf seedlings of the susceptible cultivar were uniformly inoculated in a settling tower with (uredos) spores of brown rust (*Puccinia recondita f. sp. tritici*) using a density of 345±30 spores per cm² (in one type of experiment 3 leaf seedlings were used). After 24 h in controlled conditions conducive to infection, seedlings were transferred into the growth room for incubation. The experiments lasted from the 5th to the 20th day after inoculation.

**Experimental protocol:** Four treatments were considered: control, water stress (WS), fungal stress (FS), and double stress (WS×FS). The water-stressed treatment started the second day after the inoculation date. Water stress was obtained by applying only 30 % of daily transpiration measured on control plants; FS plants were irrigated according to the water loss of the previous day. In these four treatments, measurements were taken 4 d after inoculation (before sporulation), 11 d after inoculation (just after sporulation), and 18 d after inoculation. Physiological measurements determined plant growth, and water potential and gas exchange of leaves. By measuring the number of lesions per square cm, the disease could be assessed from the 5th d (just before measurements of leaf gas exchange) to the 22nd d after inoculation.

**Plant growth and leaf water potential measurements:** Dry matter was obtained by weighing individual plants dried at 80 °C for 72 h. Using an optical leaf area meter (*Li-Cor, Lincoln, NE, USA*), leaf area was measured on the 5th inoculated leaf, the 7th leaf (appearing before sporulation on the 5th leaf), and the 8th leaf (appearing after lesion appearance on the 5th leaf). During the experiment, the predawn leaf water potential (Ψₑₑ) of 6 last fully developed leaves from 6 different pots was measured, 2 h before light start-up, using a Scholander type pressure chamber (*PMS Instruments, Corvallis, OR, USA*).
$P_N$ and $g_s$ in relation to disease and/or water stress: CO$_2$ and H$_2$O exchange were measured with a Parkinson leaf chamber connected to a CO$_2$ analyser (ADC, type LC43, Hoddesdon, UK). This transient open gas exchange system allowed simultaneous estimate of $P_N$ and $g_s$. It consists of a mass flow controller, a leaf chamber (where air temperature, humidity, and radiation are measured), an infrared gas analyser, and a data logger. The incoming air was furnished by a compressed air cylinder, the CO$_2$ concentration of which was 370 $\mu$mol mol$^{-1}$. The individual $P_N$ and $g_s$ measurements took less than 1.5 min. $P_N$ measurements started on the fourth day after inoculation. They were made each day 4 to 5 h after start of light period on leaves that were well exposed to radiation (10 leaves in 10 different pots for each of the four treatments). The range of incident photosynthetic photon flux (PPFD) density was 550-650 $\mu$mol m$^{-2}$ s$^{-1}$ inside the leaf chamber.

On leaves with lesions, $g_{H_2O}$ values quantified water transfer rate through stomata and epidermal lesions. To calculate $g_{H_2O}$, it was assumed that the diffusion ratio (1.6) between water and CO$_2$ was identical for both pathways. To calculate $C_i$, it was assumed that there was no obstacle to CO$_2$ diffusion in the intercellular spaces. Using this assumption, conductance measurements could be compared between the 4 treatments.

$P_N$ response curves under controlled irradiance and CO$_2$ concentration: At a given CO$_2$ concentration, the relationship between $P_N$ and incident radiation provides one way to characterise the plant responses to fungal and/or water stress (Fig. 1A). The corresponding curves use a non-rectangular hyperbola with 4 parameters: maximal radiation use efficiency (MRUE), dark respiration rate ($R_o$), convexity index (m), and maximal net photosynthetic rate ($P_{N_{\text{max}}}$). The appendix describes how changes in the four parameters were modelled against plant water status expressed by predawn leaf water potential ($\Psi_{\text{wp}}$). In Fig. 1A, points H and S represent $P_{N_{\text{max}}}$ values corresponding to control and stressed plants, respectively.

Changes in $P_{N_{\text{max}}}$ values result from stress effects on stomata, CO$_2$ fixation, and respiration. To separate these 3 types of action, a response curve of $P_N$ versus $C_i$ was obtained by varying the air CO$_2$ concentration ($C_i$) under saturating irradiance (Fig. 1B). Beside the general situation of change conducive to the reduction in $P_{N_{\text{max}}}$ under non-limiting PPFD (from H to S in Fig. 1B), there are three more specific situations: (1) from H to Sg, a decrease in $g_{\text{HCO}_2}$ with no modification of the response curve $P_{N_{\text{max}}}(C_i)$; (2) from H to Sm, a decrease in $P_{N_{\text{max}}}$ with no modification of $g_s$; (3) an increase in respiration (photospiration and $R_o$) rates with enhancement of the CO$_2$ compensation concentration $\Gamma$ (from $\Gamma_H$ to $\Gamma_S$). In all three situations, a decrease in $P_{N_{\text{max}}}$ corresponds to either decreasing $C_i$ due to stomata closure, or increasing $C_i$ owing to non-stomatal effects, or enhanced $R_o$.

Measurements of $P_N$ and $g_s$ were made using a portable LI-6400 photosynthesis system (Li-COR, Lincoln, NE, USA). This instrument allows generate reproducible conditions of irradiation, temperature, relative humidity, and CO$_2$ concentration. Estimated variables are $P_N$, $g_{\text{HCO}_2}$, and $C_i$ (equal to $C_s - P_N/g_s$ corrected according to Caemmerer and Farquhar 1981). Response curves for $P_N$ versus PPFD were established for 7 irradiances (0, 60, 120, 200, 500, 1, 200, and 5, 500 $\mu$mol m$^{-2}$ s$^{-1}$) under an air CO$_2$ concentration of 350 $\mu$mol mol$^{-1}$. Response curves for $P_N$ versus $C_i$ were obtained for 7 values of $C_s$ (350, 300, 200, 150, 100, 50, 0 $\mu$mol mol$^{-1}$) under saturating irradiance (1,500 $\mu$mol m$^{-2}$ s$^{-1}$). Each response curve to irradiance and CO$_2$ concentration was based on measurements made during 9 min per leaf.

Statistical analysis: The irradiance response curves of leaf photosynthesis were analysed using NLIN procedure of the Statistical Analysis System (SAS 1988). The appendix describes individual steps of this analysis. Except for photosynthesis response curves, all values shown are means of replicates (1 replicate = 1 leaf taken from one pot; number of replicates: 6 for $\Psi_{\text{wp}}$, 10 for photosynthesis and stomatal conductance ADC measurements, 12 for measurements of disease severity, leaf area, and plant growth). In most cases, variability estimates are given by the confidence interval for $p = 0.05$. The comparison of means was made using the Student's t-test between two treatments and the multiple range Duncan's test between 4 treatments using the ANOVA procedure of the Statistical Analysis System (SAS 1988).

Results

Growth: Both types of stress altered the dry matter dynamics. In the chosen growing conditions, a similar growth reduction was observed for WS and FS applied independently. The combination of both stresses reduced the plant dry matter by 22%, which corresponds to the sum of reductions caused by WS alone (13% reduction) and infection alone (8% reduction). In the FS treatment, the leaf area reduction was similar for the three types of leaves ($5^\text{th}$, $7^\text{th}$, or uninfected top leaves appearing on inoculated leaves after the beginning of sporulation) (Table 1). In comparison to control plants, leaf area reduction was between 14 and 17 %. In the WS treatment, leaf area reductions were 19, 35, and 51 % for the $5^\text{th}$, $7^\text{th}$, and uninfected top leaf, respectively. In the WS×FS treatment, the leaf area reduction was 37 and 47 % on the $5^\text{th}$ and $7^\text{th}$ leaves, respectively. These reductions corresponded roughly to the sum of leaf area reductions obtained on plants submitted to the two types of stress ap
Table 1. Leaf area [cm²] for the four treatments: control, fungal stress (FS), water-stress (WS), and double stress (WS×FS) for three leaves categories: 5th, 7th, and non-inoculated top leaf. Means ± SE.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>FS</th>
<th>WS</th>
<th>FS×WS</th>
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<tr>
<td>5th leaf (n = 22)</td>
<td>15.94±2.79</td>
<td>13.73±2.21</td>
<td>12.93±2.74</td>
<td>10.05±1.84</td>
</tr>
<tr>
<td>7th leaf (n = 14)</td>
<td>17.40±3.19</td>
<td>14.66±2.02</td>
<td>11.38±1.91</td>
<td>9.29±1.13</td>
</tr>
<tr>
<td>Top leaf (n = 14)</td>
<td>12.93±2.44</td>
<td>10.79±1.74</td>
<td>6.33±0.76</td>
<td>8.50±0.83</td>
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</table>

Fig. 1. Conceptual framework for examining (A) leaf net photosynthetic rate (Pₚ) responses for healthy (H) or stressed (S) plants to photosynthetic photon flux density (PPFD) under an air CO₂ concentration of 350 μmol mol⁻¹ with corresponding changes in maximal net photosynthetic rate (Pₚmax), maximal radiation use efficiency (MRUE), and dark respiration rate (Rₛ), or (B) response of Pₚmax to intercellular CO₂ concentration (Cᵢ) under a given PPFD (1 500 μmol m⁻² s⁻¹). Both situations, H and S, are shown with corresponding changes in the CO₂ compensation concentration for Pₚ (F) and in epidermal CO₂ conductance (gₑ) between air CO₂ concentration (Cₐ) and Cᵢ.

Fig. 2. (A) Time change in mean number of brown rust lesions per unit [cm²] of inoculated leaf area in inoculated stressed (●) and irrigated (●) plants. Each point is the mean of 16 replicates; vertical bars represent the standard error. (B) Time change in predawn leaf water potential (Ψₑₑ) for young healthy wheat plants (○), plants subjected to rust infection (●) or water stress (△), and both infection and water stress (●). Each point is the mean of six replicates ± one standard error. Open symbols correspond to measurements on days before sporulation; closed symbols correspond to measurements on days after the start of sporulation.

Disease-water relationships: On both FS and WS×FS treatments, lesions appeared on the 8th day after inoculation (Fig. 2A). The number of lesions increased in relation to the number of infection cycles on inoculated leaves. In the WS×FS treatment, the lesion number increased gradually until the 19th d, and reached an asymptotic value slightly greater than 60 lesions per cm². This threshold was partially due to the method of measurement, because the lesions tended to coalesce. The lesion number in the WS×FS treatment was always at least twice that of the FS treatment. The water status of control plants did not change significantly from day 12 to day 19 (Fig. 2B). In contrast, on the 12th d after inoculation, inoculated watered plants showed a significant change in the water status in comparison to control plants. The leaf water potentials in the WS and FS treatments were similar. In both treatments, water stress occurrence was indicated by predawn leaf water potential below ~0.3 MPa (Hsiao 1973). Under the chosen growing conditions, fungal development and the increase in water stress were concomitant in the WS×FS treatment. Owing to the added effects of both FS and WS, Ψₑₑ was significantly lower than in the two single stress treatments.

When urea appeared (9 d after inoculation), rust infected leaves showed a strong reduction in predawn leaf water potential: on diseased plants, Ψₑₑ were ~0.37 (FS) and ~0.50 MPa (WS×FS), significantly lower than ~0.25
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MPa (control) and −0.41 MPa (WS) in healthy plants. At the end of the experiment (19–22 d after inoculation), WS and FS plants showed a similar Ψwp. Nevertheless, Ψwp of the FS treatment was significantly higher than that of the WS treatment (−0.44 MPa and −0.48 MPa, respectively). The lowest Ψwp was obtained in the WS+FS treatment (−0.63 MPa) exhibiting the highest lesion area density. Due to similar number of lesions on day 12 in the WS+FS treatment and day 19 in the FS treatment, the Ψwp observed in the WS+FS treatment was significantly lower.

From day 5 to day 19 after inoculation, i.e., before and after sporulation, Ψwp was always slightly lower in the treatment with five inoculated leaves (Table 2).

Table 2. Time variation of predawn leaf water potential (PWLP, MPa) in relation to the infected leaf area (3 or 5 inoculated bottom leaves) by Puccinia recondita. Means ± SE (n = 6).

<table>
<thead>
<tr>
<th>Time after inoculation [d]</th>
<th>Ψwp (3 leaves)</th>
<th>Ψwp (5 leaves)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>−0.20±0.02</td>
<td>−0.26±0.02</td>
</tr>
<tr>
<td>12</td>
<td>−0.34±0.02</td>
<td>−0.39±0.02</td>
</tr>
<tr>
<td>19</td>
<td>−0.38±0.02</td>
<td>−0.45±0.03</td>
</tr>
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</table>

The gas exchange: The control treatment (Fig. 3A) showed minor variations in the four parameters with the 5th leaf: dark respiration (Rd), maximal radiation use efficiency (MRUE), convexity (m), and PNmax. In the FS treatment (Fig. 3B), two groups of plants could be distinguished according to sporulation occurrence. In non-sporulating plants, the irradiance response curves were similar, but in sporulating plants PN was reduced. In the WS treatment (Fig. 3C) there was also a continuous reduction in both MRUE and PNmax. In the WS+FS treatment (Fig. 3D) two categories of response curve were observed: before sporulation, a continuous decrease in PN, similar to the WS treatment, and after sporulation a very strong decrease in PNmax.

The variations in PN versus Cc under saturating irradiance (1 500 μmol m⁻² s⁻¹) allowed assess the magnitude of changes in PNmax (between 21.2 μmol m⁻² s⁻¹ and zero, Fig. 4). In control plants (Fig. 4A), PN decreased slightly over time and Ψc increased slightly. In the FS treatment, before sporulation, the same eco-physiological behaviour was observed for both PN and Ψc, with a concomitant decrease in gCO₂ (Fig. 4B). Upper curves showed a relatively constant Cc corresponding to Cc of 350 μmol m⁻² s⁻¹. When lesions were well developed (lower curve in Fig. 4C), Ψc and gCO₂ increased, the value of gCO₂ becoming close to its initial value. These changes corresponded to a noticeable Cc increase. In the WS treatment (Fig. 4C), a progressive PN reduction and stomata closure were concomitant with Cc, being approximately constant and unmodified in comparison to the control treatment. In the WS+FS treatment (Fig. 4D) two types of response curve were observed before and after start of sporulation. Before sporulation the leaf PN response was comparable to that of the WS treatment with concomitant PN decrease and stomata closure. Under the chosen availability of soil water, there was no change in the calculated Cc, nor was the Ψc value modified. After sporulation, a strong increase was noticed for both Ψc and Cc (as in the FS treatment) with a correlating decrease in gCO₂.

In control plants (Fig. 5A), the ranges of PN were comparable for newly formed leaves (after the sporulation date on infected plants) and 5th leaves (Figs. 5A and 4A). For these two categories of leaves, water stress determined a similar decrease in both PN and gCO₂ with no change in Ψc (Figs. 5C and 4C). In contrast, for newly

Fig. 3. Relationships between net photosynthetic rate (PN) and photosynthetic photon flux density (PPFD) for A) healthy wheat leaves (■●), B) infected leaves (FS, ○●), C) water-stressed leaves (WS, Δ●), and D) double stressed leaves (WS+FS, ◊●). Open symbols correspond to measurements on days before sporulation; closed symbols correspond to measurements on days after sporulation start.

Fig. 4. Relationships between net photosynthetic rate (PN) and intercellular CO₂ concentration (Cc) on the 5th leaf of wheat plants for the four treatments: control (A), fungal stress (B), water stress (C), and fungal-water stress (D). The same symbols as in Fig. 3. The response curves are fitted to a third-order polynomial equation. The epidermal conductance for CO₂ (gCO₂) is shown as the line linking Cc and the corresponding point (PN, Cc). On each of the 4 figures, thick lines represent response curves of control plants.
formed FS leaves, there was a decrease in $P_N$ with stable $g_s$ and, therefore, an increase in $C_i$ (Fig. 5B). For double stressed plants (WS+FS; Fig. 5D), stomata closure was observed (strong decrease in $g_s$) but both $\Gamma$ and the initial slope of the $P_N$-$C_i$ response curve remained unmodified.

To understand how changes in water status induced by rust infection could alter gas exchange, the $P_N$-PPFD curve was modeled analytically using the non-rectangular hyperbola whose parameters were estimated against changes in $\Psi_{wp}$ for the four treatments (Table 3). MAP$_{max}$ and MRUE$_{max}$ values were not significantly different from values estimated for control plants [2.4±0.2 μmol (CO$_2$) m$^{-2}$ s$^{-1}$ compared to the confidence interval 22.0-24.4 μmol(CO$_2$) m$^{-2}$ s$^{-1}$ for MAP$_{max}$ and confidence interval 0.067-0.075 mol(quantum) mol$^{-1}$CO$_2$ compared to 0.073-0.09 for MRUE$_{max}$]. Minimal value of $m$, $m_{min}$, was fixed to 0.41 for control plants according to the confidence interval calculated as 0.36-0.46. A slope value of $-2$ for $SLR$ determined a zero value of $P_{N_{max}}$ for a $\Psi_{wp}$ of $-0.7$ MPa. The $SLR$ value indicates that MRUE decreased as $\Psi_{wp}$ decreased whereas $SLm$ value indicates that the convexity increased with $\Psi_{wp}$.

The model expresses changes in non-rectangular hyperbola parameters according to the values estimated in Table 3 and assuming mean $R_0$ of $-1.6$ μmol(CO$_2$) m$^{-2}$ s$^{-1}$ (Fig. 6). Though the slope of the regression line was close to 1 (0.96, $r^2 = 0.92$), note that WS+FS values are above the 1:1 line and WS values below it. $P_N$ measured in the growth chamber using LCA3 ADC diminished in response to the decrease in predawn leaf water potential derived using irradiance and $\Psi_{wp}$ measurements and the hyperbolic function (Eq. 1 in Appendix). This independent data set shows that points belong to one single response relation (Fig. 7A). This hyperbolic response is close to the linear regression proposed for change in $P_{N_{max}}$ with a nil value of $P'_{N_{max}}$ for $\Psi_{wp}$ value of $-0.7$ MPa.

![Fig. 5](image)

**Fig. 5.** Relationships between net photosynthetic rate ($P_N$) and intercellular CO$_2$ concentration ($C_i$) in healthy leaves appearing after inoculation at the top of infected plants. The same symbols as in Fig. 4.

![Fig. 6](image)

**Fig. 6.** Comparison of observed and modelled net photosynthetic rate, $P_N$, for the four treatments with observed values of the Fig. 3. The dotted line represents the linear regression between observed and modelled $P_N$ ($AP_{modelled} = 0.96 AP_{observed}$, $r^2 = 0.92$). The 1:1 line is represented. *Open symbols* for healthy leaves (control: $\bigtriangleup$; WS: $\bullet$); *closed symbols* for inoculated leaves (FS: $\bigtriangleup$; WS+FS: $\bullet$).

![Fig. 7](image)

**Fig. 7.** (A) Relationship between net photosynthetic rate, $P_N$, and predawn leaf water potential ($\Psi_{wp}$), for the four treatments. Comparison of the $P_N$ measurements made inside the growth chamber and the $P_N$ modelled under a range of PPFD between 550 and 650 μmol m$^{-2}$ s$^{-1}$. The same symbols as in Fig. 6. Each point represents the mean value of 10 leaves from different plants. Line represents the linear regression between modelled values (X) and $\Psi_{wp}$ (B) Relationships of CO$_2$ diffusion conductance ($g_s$) and predawn leaf water potential ($\Psi_{wp}$) for the four treatments; the same symbols as in Fig. 3. Each point represents the mean value of 10 leaves from different plants. Vertical bars represent the standard error.

In control plants, $g_{CO2}$ did not significantly change (Fig. 7B). From the day of inoculation to the beginning of sporulation (0), $g_s$ in FS was similar to $g_s$ in control plants. At the beginning of sporulation (9 to 14 d after inoculation), $g_s$ in FS plants was significantly higher (up to 0.5 mol m$^{-2}$ s$^{-1}$) than in control plants (0.33 mol m$^{-2}$ s$^{-1}$), then (after day 14), $g_s$ values in FS plants decreased to values similar to those of control plants. These results
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indicate that brown rust sporulation induces a transient enhancement of water logging in the FS treatment. In WS plants, \( g_s \) continuously decreased. In WS×FS plants, \( g_s \) remained close to 0.15 mol m\(^{-2}\) s\(^{-1}\). Two different ranges of \( g_s \) were observed for irrigated plants (control and FS) and water-stressed plants (WS and WS×FS). In healthy plants, \( g_s \) decreased with decreasing predawn leaf water potential. In infected plants there was a lot of scattering. In plants submitted to double stress, \( g_s \) was low (less than 0.15 mol m\(^{-2}\) s\(^{-1}\)) and stable for a range of predawn leaf water potentials between -0.3 and -0.7 MPa.

Table 3. Parameter estimates of the non-rectangular hyperbola model of the photosynthetic irradiance response curve for the 4 treatments, control, water stress, fungal stress, and both stresses together. \( \text{MAP}_{\text{max}} \), maximal net leaf photosynthetic rate of unstressed plant [\( \mu \text{mol}(\text{CO}_2) \text{ m}^{-2} \text{s}^{-1} \)], \( \text{MRUE}_{\text{max}} \), maximal radiation use efficiency of unstressed plant [mol(quantum) mol\(^{-1}\)CO\(_2\)], \( m_{\text{min}} \), minimal convexity index, \( SL_{P} \) [\( \mu \text{mol}(\text{CO}_2) \text{ m}^{-2} \text{s}^{-1} \text{ MPa}^{-1} \)], \( SL_{A} \) [mol(quantum) mol\(^{-1}\)CO\(_2\) MPa\(^{-1}\)], and \( SL_{m} \) [MPa\(^{-1}\)], the slopes of the change of \( P_{\text{max}} \), MRUE, and \( m \), respectively, against the predawn leaf water potential.

<table>
<thead>
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<th>Parameter</th>
<th>Estimate</th>
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<th>Asymptotic 95% confidence interval</th>
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<tr>
<td></td>
<td></td>
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</tr>
<tr>
<td>MAP(_{\text{max}})</td>
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<td>0.605</td>
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<tr>
<td>MRUE(_{\text{max}})</td>
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<td>0.0046</td>
<td>0.0729</td>
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<tr>
<td>( m_{\text{min}} )</td>
<td>0.410</td>
<td>0.023</td>
<td>0.361</td>
</tr>
<tr>
<td>( SL_{P} )</td>
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<tr>
<td>( SL_{A} )</td>
<td>-1.223</td>
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<tr>
<td>( SL_{m} )</td>
<td>1.316</td>
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Discussion

In relation to the increase in number of brown rust lesions responsible for plant water losses, there was a \( \Psi_{wp} \) reduction in wheat leaves (Fig. 2D). On days 12 and 19, leaf rust and water stress had additive effects on \( \Psi_{wp} \) reduction: the reduction was -0.38 MPa in WS×FS treatment in comparison to -0.17 and -0.20 MPa in FS and WS treatments, respectively. Paul and Ayres (1984) made similar observations with groundsel infected by 

\( \text{Puccinia lagenophorae} \) and submitted to a post-infection drought. The combined effects of \( \text{P. lagenophorae} \) infection and drought led to a leaf water potential reduction of -1.74 MPa in comparison to healthy control plants, whereas FS and WS applied independently produced \( \Psi_{wp} \) reductions of -1.02 and -0.77 MPa, respectively.

The \( \Psi_{wp} \) response of the wheat plants depended on the number of infected leaves. With 5 infected leaves in comparison to only 3, there was a significant decrease in \( \Psi_{wp} \) corresponding to the larger diseased area on days 5, 12, and 19 after inoculation. When measuring leaf water potential during daytime hours, comparable results were obtained in barley infected by \( \text{Puccinia hordei} \) (Berrymen et al. 1991) and in sugar beets infected by \( \text{Erysiphe polygoni} \) (Gordon and Duninway 1982).

To simulate the \( P_N \)-irradiance response under single or combined leaf rust and/or infection drought stresses, a model was built using a non-rectangular hyperbolic function of radiation with \( \Psi_{wp} \)-dependent parameters. After comparison with \( \text{Li-6400} \) measurements, the \( P_N \) versus \( \Psi_{wp} \) model was tested using independent \( \text{LCA3} \) ADC measurements obtained in the growth chamber under given irradiance (500-600 \( \mu \text{mol} \text{ m}^{-2} \text{s}^{-1} \)). With this independent set of values, the relationship between measured \( P_N \) and \( \Psi_{wp} \) (Fig. 7A, bold symbols) was close to the modelled relationship based on Eq. 1 in Appendix (open symbols). The slopes of the linear regressions between measured or modelled \( P_N \) against \( \Psi_{wp} \) were 32.3 and 28.4, respectively, with corresponding \( r^2 \) coefficients of 0.87 and 0.99. Although these slope values are in the same range, they differ significantly at the 0.05 level. Compared to the \( P_N \) values measured in the growth chamber, the model output was rather satisfactory (regression slope = 0.76, \( r^2 = 0.86 \)). The differences between model and measurements might be due to non-linearity of the three model parameters against \( \Psi_{wp} \) (Eqs. 2, 3, and 4 in Appendix). This confirmed the predominant role of \( \Psi_{wp} \) as a factor controlling \( P_N \) variation. At leaf scale, a unique relationship was found between \( P_N \) and \( \Psi_{wp} \) resulting from WS, FS, or WS×FS. The \( P_N \) reductions observed for each type of stress can be explained in one situation (WS) by a concomitant reduction in \( P_N \) and \( g_s \) (Fig. 4C), in another case (FS) by a reduction in \( P_N \) with no variation in \( g_s \), but with an increase in respiratory processes indicated by a doubling of \( \Gamma \) together with a decrease in mesophyll conductance that indicates a decrease in carboxylation efficiency (Fig. 4B).

There is a controversy over the mechanisms by which stress decreases \( P_N \) and the regulation between these mechanisms (Cornic 2000). Three principal mechanisms are invoked: (1) restricted diffusion of CO\(_2\) caused by stomata closure (Bethenod et al. 1996), (2) inhibition of CO\(_2\) metabolism caused by the reduction of ribulose-1,5-bisphosphate synthesis whose intensity is shown by
mesophyll conductance, and (3) change in the ratio between oxygenase and carboxylase activities of ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBPCO) shown by variation in \( \Gamma \) (Cornic 1994).

The \( P_{n} \) decrease could not be explained by stomata closure in infected plants (Fig. 4B). \( \Psi_{wp} \) decrease revealed a dehydration in infected plants (Fig. 2). In both FS and WS*FS treatments, the observed increase of \( \Gamma \) (Fig. 4B,D) indicated the occurrence of the third mechanism with the increase of oxygenase activity of RuBPCO in response to dehydration. In all treatments, the decrease of mesophyll conductance (initial slope of the \( P_{n}/C_{i} \) response curve) was linked to \( \Psi_{wp} \) decrease, therefore no conclusion could be made concerning the role of the second mechanism to discriminate between stress effects (Fig. 4).

In the present study, the analysis of plant development over time indicated differences in leaf growth. This is consistent with the cell growth sensitivity to plant water stress (Hsiao 1973). FS caused by \( P. \) recondita and WS had additive deleterious effects on the development of leaves 5 (infected) and 7 (non-infected, Table 2) as well as on plant dry matter production. However, for whatever leaf number (5, 7, top leaf), the reduction in leaf area of FS treatment was almost constant (15%). In the WS treatment the leaf area was reduced by 19% (leaf 5) to 51% (non-infected top leaf). In the WS and FS treatments, \( \Psi_{wp} \) were similar. These results along with the differences in leaf area reduction in response to each type of stress suggest the existence of an effect compensating the water stress impact on non-inoculated leaves. Leaf expansion is one of the first processes affected by WS, therefore the growth of new leaves in the WS treatment was more strongly affected than the growth of leaves which appeared before the stress occurred. In the WS*FS treatment, the area reduction of non-infected top leaves corresponded approximately to the average of both area reductions observed on WS and FS treatments applied independently, and not to their sum as in the case of leaves 5 and 7. This observation is consistent with the existence of a compensatory effect that can be assessed by the difference of leaf area reduction (expressed as % of control treatment) between the single WS and FS treatments. Benefiting to the FS treatment, this compensatory effect was low (4.0%) on leaf 5, intermediate (18.5%) on leaf 7, and high (34.5%) on top leaf, respectively. Such effect could explain the lower reduction in the area of top leaves in the WS*FS treatment (34%) compared to the WS treatment (51%). Thus, the infected plant is partially able to resist rust infection in upper, uninocfected leaves of wheat similarly as shown by Murray and Walters (1992) for rusted broad bean.

Stomata behaviour is very different between inoculated (FS and WS*FS treatments) and non-inoculated plants (control and WS treatments). In non-inoculated plants, the \( g_{CO2} \) decrease with predawn water potential (\( \Psi_{wp} \), Fig. 7B) together with \( P_{n} \) decrease (Fig. 7A) induce a \( C_{i} \) constancy close to 200 \( \mu \)mol mol\(^{-1} \) (Fig. 4C). In inoculated plants, stomata behaviour was more erratic: after sporulation, the transient increase of \( g_{i} \) in FS treatment or the \( g_{i} \) stability in WS*FS treatment close to 0.15 mol m\(^{-2} \) s\(^{-1} \) lead to a \( C_{i} \) increase, when \( P_{n} \) decreased (Fig. 4B,D). In the FS treatment the transient increase of \( g_{i} \) stopped after 3 d and the difference in \( g_{i} \) values disappeared between irrigated treatments (control and FS) as between water stressed treatments (WS and WS*FS) even though \( \Psi_{wp} \) values were different (Fig. 7B). This is not consistent with previous works dealing with stomata closure in response to chemical messages. Such is the case for \( S. \) nodorum in wheat (Bethenod et al. 1982) and \( H. \) maydis in corn (Arntzen et al. 1973). In the case of brown rust, no specific chemical message has been discovered. Therefore the mechanical changes in diseased leaves are likely to generate a water status modification.

In conclusion, \( \Psi_{wp} \) is a pertinent indicator of plant response to a single stress (brown rust infection or soil water deficit) or a twofold stress in the chosen experimental conditions. If both brown rust infection and post-infection drought produce similar changes in \( \Psi_{wp} \), these stresses lead to a similar physiological strain (reduction in \( P_{n} \) by means of different mechanisms of gas exchange regulation) and a similar damage (reduction in plant growth).

It is helpful to invoke the concept of stress response interaction outlined by Higley et al. (1993). In the case of dry matter of infected plants, area of infected leaves, and photosynthesis, the effects of fungal infection and post-infection drought on \( P_{n} \) were additive, and no stress interaction was found. In the case of \( g_{i} \) and leaf area of non-infected top leaves, there was an interaction between the responses to fungal infection and post-infection drought.

References


PHOTOSYNTHETIC RESPONSE OF WHEAT TO A TWOFOOLD STRESS


APPENDIX

### Determination of the parameters of non-rectangular hyperbola model of photosynthetic irradiance response curve

The dependence of leaf net photosynthetic rate (*Pn*, μmol m⁻² s⁻¹) on irradiance [photosynthetic photon flux density, PPFD; μmol (quantum) m⁻² s⁻¹] is described by a non-rectangular hyperbola (Prioul and Chartier 1977, Cannell and Thornley 1998):

\[
P_n = R_0 + \{MRUE \cdot PPFD + P_{Nmax} - [(MRUE \cdot PPFD + P_{Nmax})^2 - 4 \cdot m \cdot MRUE \cdot PPFD \cdot P_{Nmax}^{0.5}] / 2 \} / m
\]

where MRUE is the quantum yield of CO₂ assimilation based on irradiance that represents the initial slope of the hyperbola, *P_{Nmax}* is the irradiance-saturated photosynthetic response of wheat to a two-fofold stress.

Because *R₀* is a result of processes other than photo-

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synthesis, our first assumption was that $R_D$ was constant with a mean value of 1.6 $\mu$mol m$^{-2}$ s$^{-1}$. In order to test the dependence of the irradiance-$P_N$ relationship to the internal water status, we used the response of the three parameters, MRUE, $P_{N_{\text{max}}}$, and $m$, to the predawn leaf water potential ($\Psi_{wp}$) in wheat (Johnson et al. 1983, Tardieu and Katerji 1991). To achieve this, we determined the maximal value of MRUE and $P_{N_{\text{max}}}$, $MRUE_{\text{max}}$, and $P_{N_{\text{max}}}$, respectively, and the minimal value of $m$ ($m_{\text{min}}$) based on existing knowledge (Hsiao 1973) for $\Psi_{wp} = 0.2$ MPa.

We assumed that MRUE and $P_{N_{\text{max}}}$ are linearly decreasing and $m$ is linearly increasing as $\Psi_{wp}$ decreases. Let $SL_R$, $SL_P$, and $SL_m$ be the slope of the linear response of MRUE, $P_{N_{\text{max}}}$, and $m$, respectively.

\begin{align*}
MRUE &= MRUE_{\text{max}} [1 - SL_R (\Psi_{wp} + 0.2)] \\
P_{N_{\text{max}}} &= MAP_{\text{max}} [1 - SL_P (\Psi_{wp} + 0.2)] \\
m &= m_{\text{min}} [1 - SL_m (\Psi_{wp} + 0.2)]
\end{align*}

We substituted MRUE, $P_{N_{\text{max}}}$, and $m$ relations in Eq. (1). Then the NLIN procedure of the Statistical Analysis System (SAS 1988) was used with the Marquardt method to determine the parameters in Eqs. 2, 3, and 4.